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# Lymphoid Cell Kinetics Under Continuous Low Dose-Rate Gamma Irradiation — A Comparison Study

by

Bessie Ruth Foster

## PURPOSE, METHODS AND FINDINGS

The objective of the current research is to make a comparison study of the effects of continuous low dose-rate gamma irradiation on cell population kinetics of lymphoid tissue (white pulp) of the mouse spleen with findings as they relate to the mouse thymus.

Experimental techniques employed include autoradiography and specific labeling with tritiated thymidine (TdR-$^{3}$H).

The problem being studied involves the mechanism of cell proliferation of lymphoid tissue of the mouse spleen under the stress of continuous irradiation at a dose-rate of 10 roentgens (R) per day for 105 days (15 weeks).

On the basis of thymidine labeling and distribution of various cell types in the thymus under continuous irradiation, it was concluded that at least four compensatory mechanisms served to maintain a near-steady state of cellular proliferation:

1) an increase in the proportion of PAS-positive cells which stimulate mitotic activity
2) "maturation arrest" of proliferating and differentiating cells which tend to replenish the cells damaged or destroyed by irradiation
3) an increase in the "proportion of cells proliferating," and
4) an increase in the proportion of precursor cells.

Data relative to thymus and spleen weights under continuous irradiation have been presented and discussed in the Final Technical Report associated with Grant NGR-19-011-008, submitted to NASA Scientific and Technical

Information Facility October 4, 1974.

This report deals with findings as they relate to distribution of cell types and thymidine labeling in the lymphoid component of the spleen compared to previous findings observed in the thymus.

Distribution of Cell Types and TdR-$^{3}$H Labeling

One hundred, 29-day old $BCF_1$ male mice were irradiated at a dose rate of 10R per day for 105 days. Four mice were removed from the irradiation unit and sacrificed one hour following an intraperitoneal injection of TdR-$^{3}$H at various time intervals until 105 days had elapsed. Standard techniques were used to dissect thymuses and spleens and to process the tissue through autoradiography with subsequent staining and microscopic examination.

One hundred unirradiated mice of the same strain, sex, and age as the irradiated group were processed in a similar manner. These mice served as controls.

Each data point on any given graph represents an average on 4 animals, and at least 1000 thymus or spleen cells (white pulp) were counted and categorized per microscopic examination per animal. Cells were classified on the basis of stain hue and morphology as PAS-positive reticular cells, non-PAS positive reticular cells, and lymphocytes.

Figrue 1 illustrates the distribution of PAS-positive reticular cells in the thymus and spleen under continuous irradiation. Although the total number of PAS-positive cells observed was small, a significant increase in their abundance occurred in the irradiated thymuses during the latter phase of the irradiation period. There was also an increase in the proportion of PAS-positive cells in the irradiated spleen, but only during the initial phase of the irradiation period.

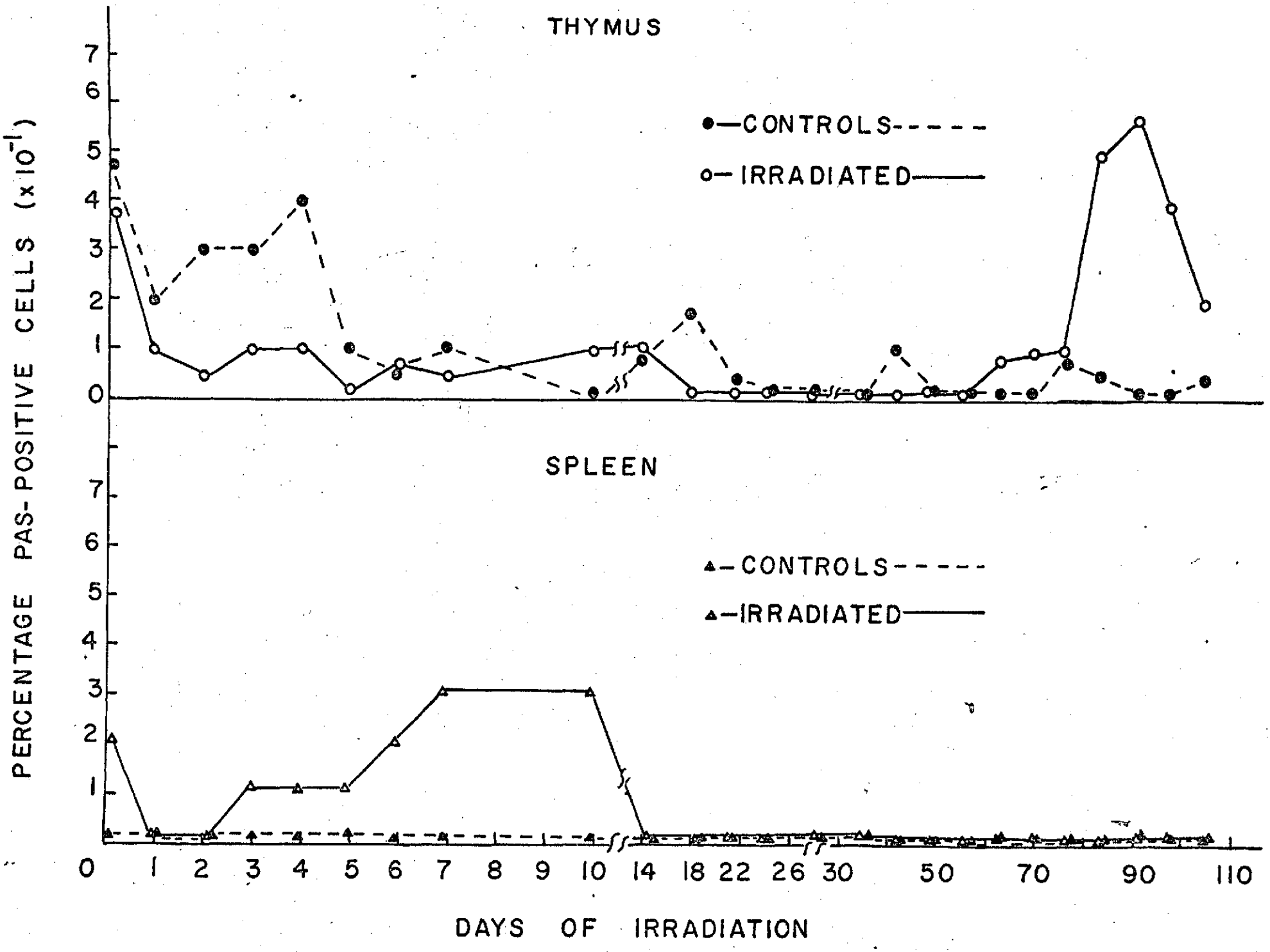
THYMUS
SPLEEN
PERCENTAGE PAS-POSITIVE CELLS (x10⁻¹)
●—CONTROLS- - - - -
o— IRRADIATED——
▲—CONTROLS- - - - -
▲—IRRADIATED——
0 1 2 3 4 5 6 7 8 9 10 14 18 22 26 30 50 70 90 110
DAYS OF IRRADIATION

Figure 1

PAS-positive reticulum cells in the thymic cortex and mitoses in the lymphoid cells in contact with the PAS cells have been observed (Goodwin, 1939; Gordon, 1955; Metcalf and Ishidate, 1962; Metcalf, 1964). These cells are reported to be most marked in the thymuses of non-neoplastic, high-leukemia AKR mouse strains, and to a lesser degree in the thymuses of other mouse strains. In the thymus of high-leukemia AKR mouse strains there were three times as many mitoses in lymphocytes surrounding these cells as in lymphocytes elsewhere in the thymus (Metcalf and Ishidate, 1962; Metcalf, 1964). These changes were interpreted as a "triggering process" induced by PAS-positive reticulum cells stimulating mitosis among lymphocytes coming in contact with them, and thereby regulating lymphopoiesis.

It has been reported previously (Final Technical Report associated with Grant NGR-19-011-008, October 4, 1974), that the response of both the thymus and spleen to continuous low dose-rate irradiation is multiphasic. That is, alternating periods of steady state growth, followed by "collapse," which in turn is followed by another period of homeostasis. Further, that the spleen is affected to a greater extent with shorter periods of steady-state growth than exhibited by the thymus.

The absence of PAS-positive cells in the spleen from about two weeks of irradiation throughout the remainder of the 105-day irradiation period suggests that an increased stimulus for mitotic activity as evidenced by the presence of PAS-positive cells was not operative in the splenic lymphoid tissue at a similar time interval nor to the same extent at that exhibited in the thymus.

Data on non-PAS positive reticular cells are presented in Figure 2. These data illustrate that the proportion of non-PAS positive reticular

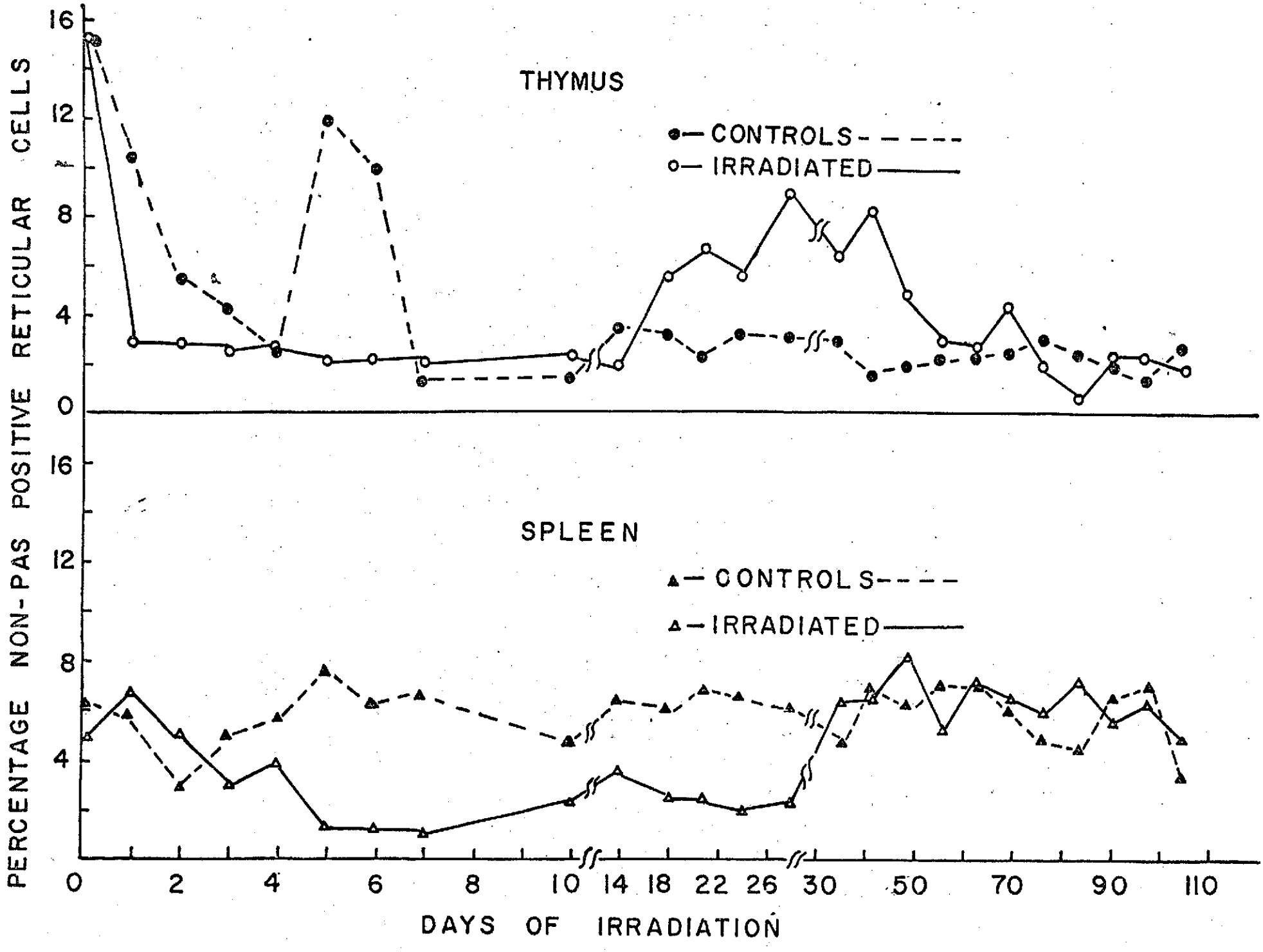
THYMUS
CONTROLS
IRRADIATED
SPLEEN
CONTROLS
IRRADIATED
PERCENTAGE NON-PAS POSITIVE RETICULAR CELLS
DAYS OF IRRADIATION
0
2
4
6
8
10
14
18
22
26
30
50
70
90
110
16
12
8
4
0

Figure 2

cells was generally smaller in irradiated thymuses and spleens at the onset of irradiation, compared to non-irradiated controls. After the first few weeks of irradiation, there was an increase in the proportion of non-PAS reticular cells in the irradiated thymus. However, this occurrence was not observed in the spleen until about 6 weeks of irradiation, and the increase in the proportion of non-PAS reticular cells in irradiated spleens was only slightly greater than that observed in comparable controls.

Since reticular cells of lymphoid tissue are precursor cells which give rise to lymphocytes, these findings suggest that another compensatory mechanism which serves to re-establish the near-steady state of cellular proliferation in the thymus and spleen under continuous irradiation is a state of "maturation arrest" among non-PAS positive reticular cells. That is, a greater proportion of the lymphoid cell population remains in the "progenitor" or "precursor" state under continuous irradiation, thereby, producing cells which compensate for those that are damaged or destroyed by radiation.

As with the findings on PAS cells in the thymus and spleen, the increase in the proportion of non-PAS reticular cells was observed at different time intervals and to a lesser extent in irradiated spleen tissue compared to the thymus.

Thymidine labeling in non-PAS reticular cells is illustrated in Figure 3. Generally, there was a smaller percentage of labeling among irradiated non-PAS reticular cells in both the thymus and the spleen, with the exception of about a two-week period in the spleen (day 14 - day 28). Since there was generally less labeling in irradiated non-PAS reticular cells in the thymus and spleen, these data suggest that the reticular cell population did not increase its capacity to proliferate.

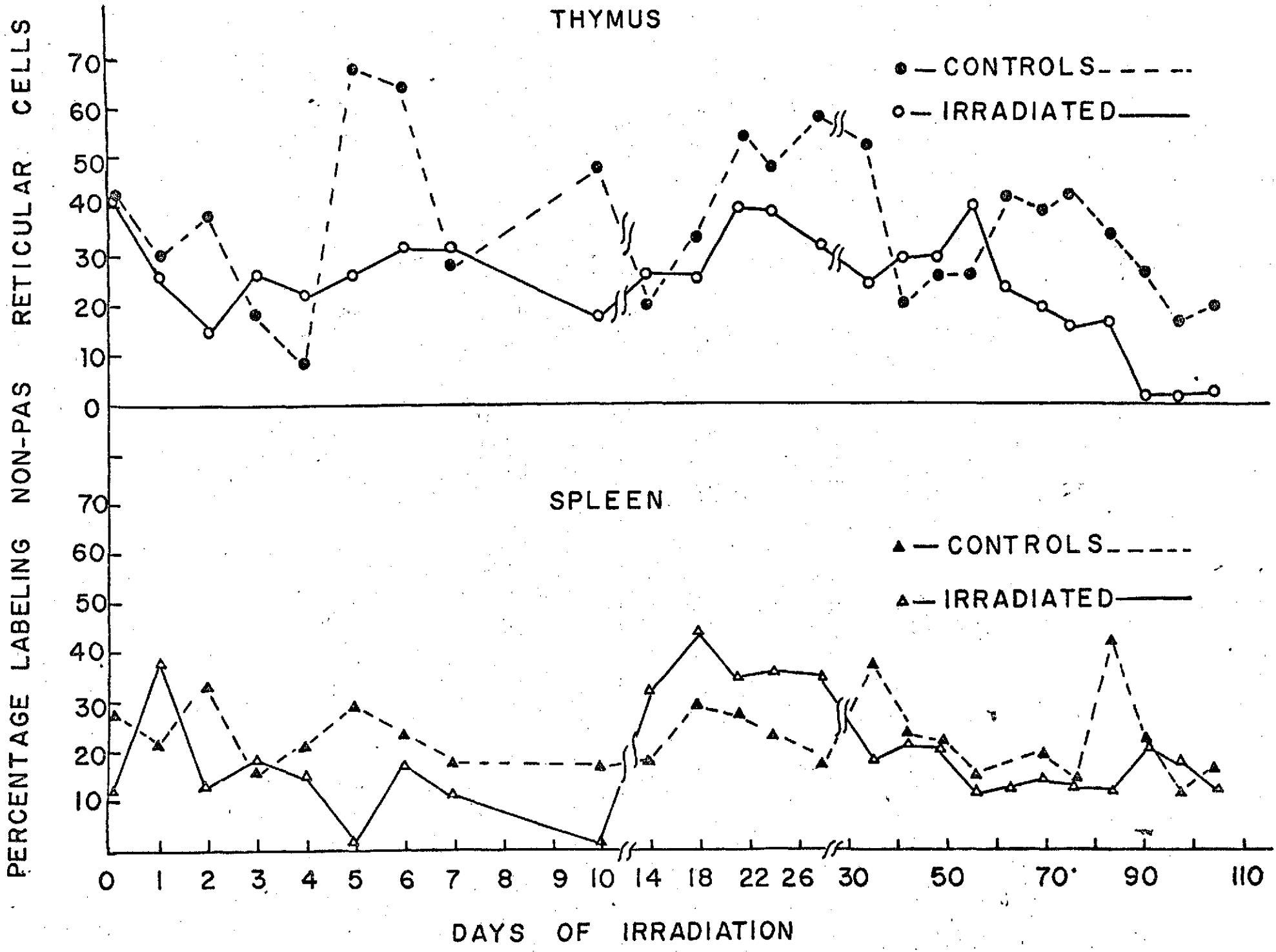
THYMUS
CONTROLS
IRRADIATED
SPLEEN
CONTROLS
IRRADIATED
PERCENTAGE LABELING NON-PAS RETICULAR CELLS
0
10
20
30
40
50
60
70
0 1 2 3 4 5 6 7 8 9 10 14 18 22 26 30 50 70 90 110
DAYS OF IRRADIATION

Figure 3

The proportion of lymphocytes and labeling among lymphocytes are illustrated in Figure 4 and Figure 5, respectively. The percentage of lymphocytes in both the thymus and spleen averaged about 95% in irradiated and control tissue. Therefore, there was no significant change in the proportion of lymphocytes under continuous irradiation.

Thymidine labeling among lymphocytes was greater in the irradiated spleen at the onset of irradiation compared to thymus tissue. After about three weeks of irradiation, there was a significant increase in thymidine labeling in the irradiated thymus, with very little labeling in comparable spleens. Since thymidine labeling suggests DNA synthesis which in turn suggests that cells are in preparation for division, a third compensatory mechanism which enables the lymphoid tissue of the thymus and spleen to maintain a near-steady state of cellular proliferation under continuous irradiation may be an increase in the "proportion of cells proliferating."

Here, too, a similar pattern of comparison is borne out. That is, a similar phenomenon occurs in both the thymus and spleen, but at different time intervals and to a different degree in the spleen. For example, there was only about 4% labeling among lymphocytes of the irradiated spleens compared to about 26% labeling among lymphocytes in irradiated thymuses averaged over the 105-day irradiation period.

Labeling in the total population is illustrated in Figure 6. Generally, there was a greater percentage of labeling in the irradiated spleen during the first few weeks of irradiation compared to the irradiated thymus. Thereafter, labeling in irradiated and control thymuses and spleens paralleled each other, with labeling in the spleen taking on lesser values.

Lastly, a comparison of findings observed in the thymus and spleen under continuous irradiation is found in Table 1. An average was taken

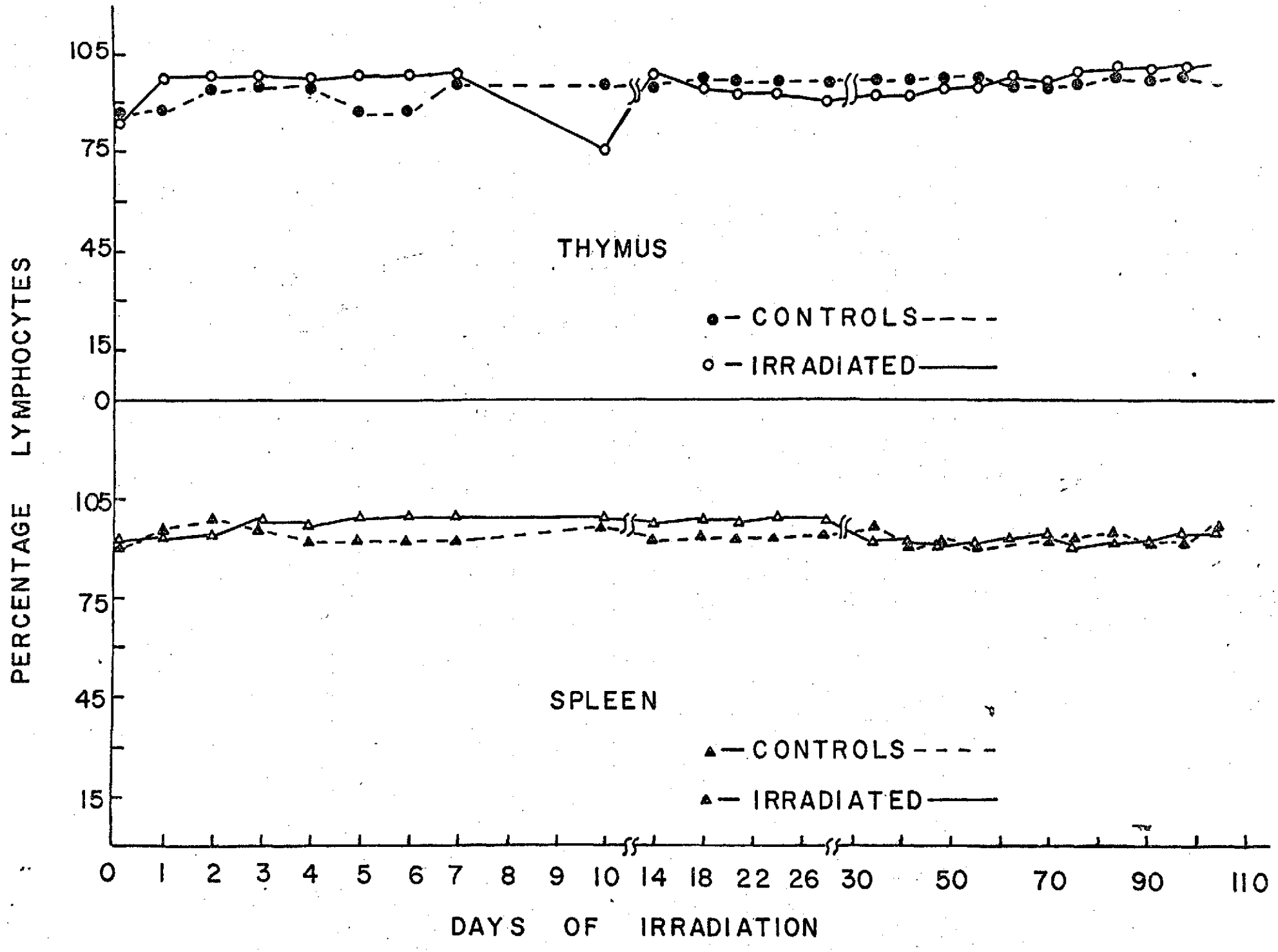
105
75
45
15
0
THYMUS
CONTROLS
IRRADIATED
105
75
45
15
SPLEEN
CONTROLS
IRRADIATED
PERCENTAGE LYMPHOCYTES
0 1 2 3 4 5 6 7 8 9 10 14 18 22 26 30 50 70 90 110
DAYS OF IRRADIATION

Figure 4

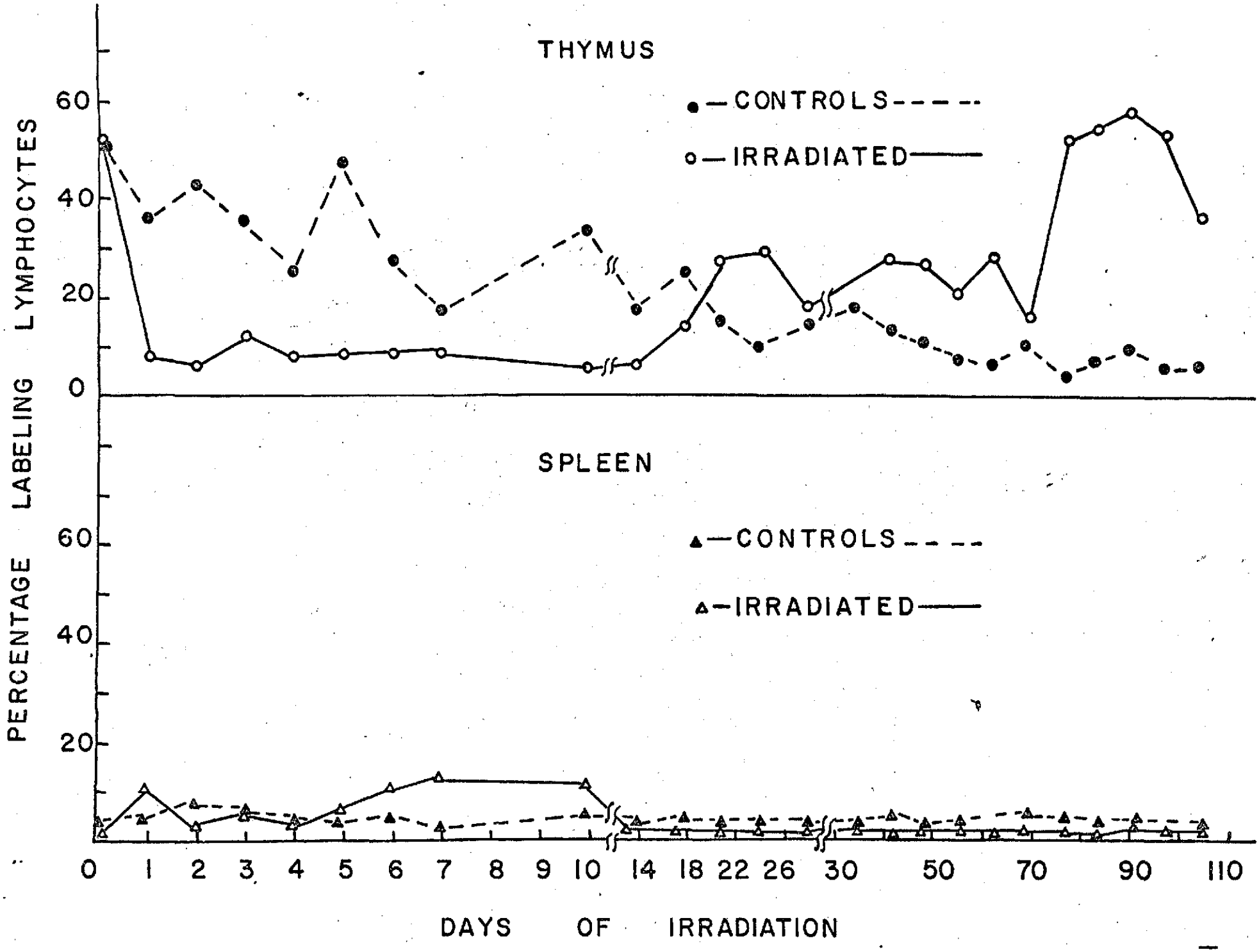
THYMUS
CONTROLS
IRRADIATED
60
40
20
0
SPLEEN
CONTROLS
IRRADIATED
60
40
20
PERCENTAGE LABELING LYMPHOCYTES
0 1 2 3 4 5 6 7 8 9 10 14 18 22 26 30 50 70 90 110
DAYS OF IRRADIATION

Figure 5

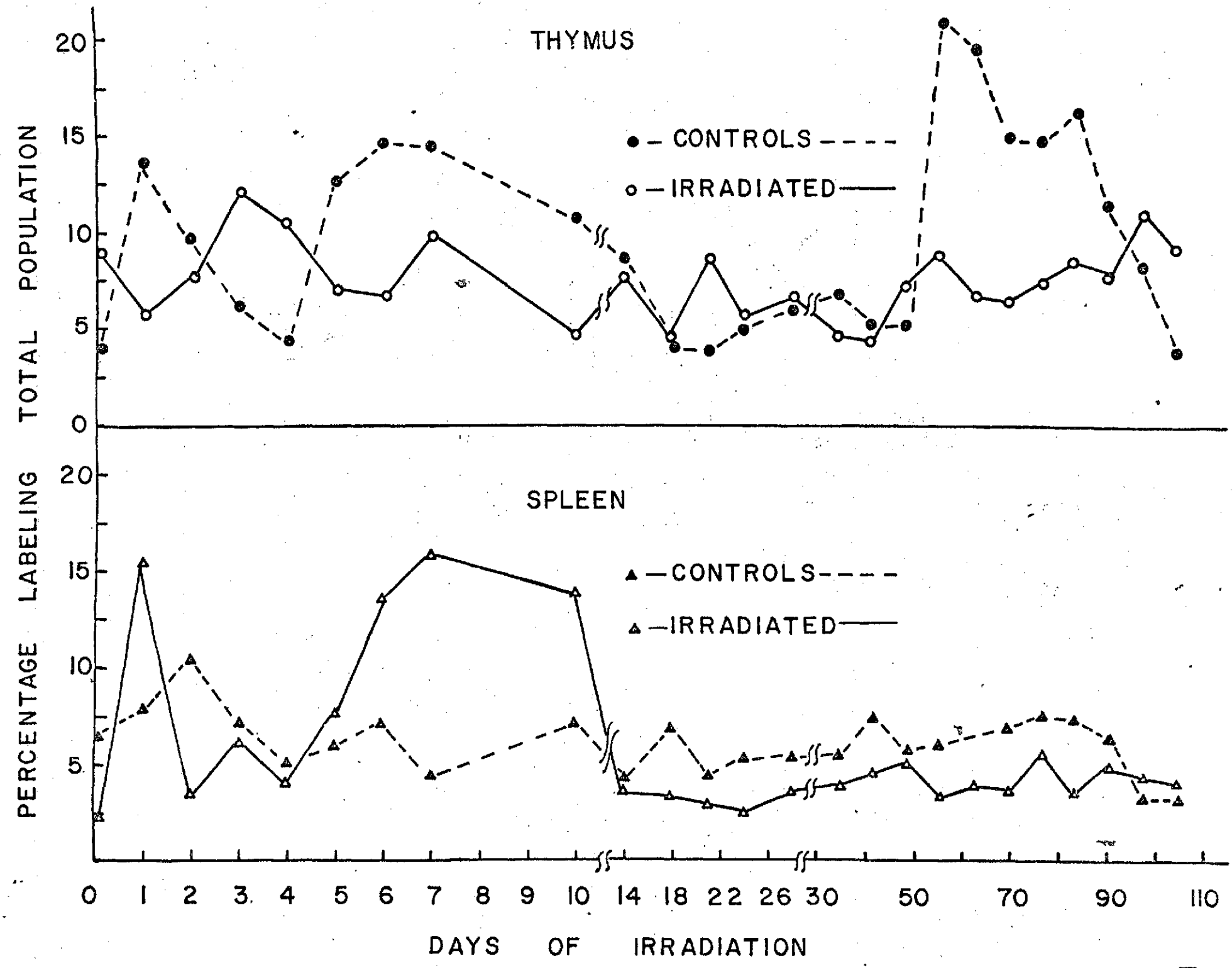
THYMUS
CONTROLS
IRRADIATED
SPLEEN
CONTROLS
IRRADIATED
PERCENTAGE LABELING TOTAL POPULATION
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 14 18 22 26 30 50 70 90 110
DAYS OF IRRADIATION

Figure 6

TABLE 1

COMPARISON OF FINDINGS OBSERVED IN THE THYMUS AND SPLEEN UNDER CONTINUOUS IRRADIATION (AVERAGED OVER 105-DAY IRRADIATION PERIOD)

| Cell Type or Parameter | Thymus | | Spleen | |
|---|---|---|---|---|
| | Percentage of Population or Category | | Percentage of Population or Category | |
| | Controls | Irradiated | Controls | Irradiated |
| PAS Positive Reticular Cells | 0.11 | 0.12 | 0.00 | 0.05 |
| Non-PAS Positive Reticular Cells | 4.22 | 4.06 | 5.89 | 4.54 |
| Labeling Non-PAS Positive Reticular Cells | 37.01 | 25.06 | 23.70 | 20.16 |
| Lymphocytes | 95.74 | 95.22 | 94.00 | 95.48 |
| Labeling Lymphocytes | 19.97 | 26.30 | 3.93 | 3.96 |
| Labeling Total Population | 10.00 | 7.59 | 4.86 | 4.76 |

on each parameter studied over the entire 105-day irradiation period. These averages suggest that there were about twice as many PAS-positive cells in irradiated thymus tissue compared to spleens. Among non-PAS reticular cells, labeling in non-PAS cells, and the proportion of lymphocytes; findings in thymus and spleen tissue were comparable. Labeling in thymus lymphocytes was about 6 to 7 times the values observed in spleens, and labeling in the thymus population averaged about twice that observed in spleen tissue.

## PROGRESS REPORT

Two studies pertaining to the current research are yet to be carried out, one on the distribution of lymphocyte classes and their proliferative capacity, and an estimation on the cell cycle time. Microscopic examination on the former study is in progress, while tissue sections for the latter are being prepared for autoradiography.

At our current pace, the research should be completed during the proposed period of performance.

## SUMMARY AND CONCLUSIONS

On the basis of distribution of cell types and thymidine labeling, it is concluded that response of both the thymus and spleen to continuous irradiation is similar, but the compensatory mechanisms are operative at different time intervals for the two tissues, and that spleen cells are stimulated to proliferate to a lesser extent than thymus cells.

## COMMENTS

No further comments are pertinent at this time.

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Metcalf, D. and Ishidate, M., Jr. (1962). Austral J Exp Biol, 40, 57.